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Leaf macro- and micro-morphological altitudinal variability of *Carpinus betulus* in the Hyrcanian forest (Iran)

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Abstract: We investigated the altitudinal variation of *Carpinus betulus* L. in the Hyrcanian forest using leaf macro-morphological and micro-morphological traits. We collected a total of 1600 leaves from two locations. In each location, we sampled six populations along an altitudinal gradient ranging from 100 m to 1,150 m. We found that trees in the higher elevations have smaller leaf lamina than those in the lower elevations. In contrast, leaf mass per area was high at low altitudes and increased newly at the higher ones. Stomatal dimension was negatively correlated with elevation, while stomatal density was positively correlated with elevation. We also found that two transects showed the same plasticity trend. Leaf area showed the highest plasticity, while the number of veins showed the lowest plasticity. This study shows that altitude, and related temperature and rainfall, represents an important driving force in *Carpinus betulus* leaf morphological variation. Moreover, our results suggest that leaf area, leaf mass per area and stomatal density could influence the species responses to different ecological conditions.

Keywords: adaptation; elevational transect; leaf; mountain forest; stomata

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Introduction

In Iran, the Alborz Mountains, between the Caspian Sea and the Iranian Plateau, create an environment extremely propitious to the growth of a unique flora in the Hyrcanian Forest. The Hyrcanian Forest, most closely to forest in a Euro-Siberian zone, is rich in broadleaf species (about 80 tree and 50 shrub species), while only three to four conifer species. Ecological conditions such as soil, temperature, rainfall and solar radiation vary considerably and visibly with increasing altitudes (Geeske et al. 1994; Lomolino 2001; Austrheim 2002; Wang et al. 2003; Körner 2007). In Hyrcanian forest, low-altitude plants have to withstand the unfavourable climatic conditions of dry summers, with high temperatures, harsh radiation and scant precipitation. On the other hand, high altitude plants have to face the adverse winter conditions with low temperatures accompanied by high irradiance. These diverse climatic conditions result in different plant morphological adaptations.

High-altitude species tend to be morphologically and physiologically distinct from closely related species from lower altitudes. Altitude has a major effect on leaf morphology and physiology within a species (Körner 1999). In general, leaf morphological traits, such as leaf length, width and area, are negatively associated with increasing altitude (Körner et al. 1986). In contrast, leaf thickness increases with increasing altitude (Körner et al. 1989; Roderick et al. 2000). Therefore, a study of the relationships between altitude and plant morphology is extremely useful in ecological studies and could be considered by plant physiologists, ecologists and palaeo-botanists. On the other hand, this high variability can lead to some ambiguities in plant taxonomy and makes difficult a comprehensive understanding about the possibility of acclimation and adaptation of a species to particular environmental conditions. Yousefzadeh et al. (2010) reported limited information about leaf variability of *Parrotia persica* along an elevational gradient in the Hyrcanian forest, but it seems this species was a poor choice because *P. persica* doesn't

occur across many elevations. Hornbeam (*Carpinis betulus*) is one of the most valuable tree species in the Hyrcanian forest and occurs across many elevations from the coastal plain at sea level to an altitude of around 1800 m in *Quercus-Carpinetum* and *Parrotia-Carpinetum* communities (Sagheb Talebi et al. 2003). Such a wide range of ecological conditions could foster the formation of different ecotypes and/or botanical varieties. Sabety (2001), for example, reported *Carpinus betulus* var. *betulus* Browicz 1972, *Carpinus betulus* var. *carpinizza* (Host) Neilr, *Carpinus betulus* var. *parva* Radde-Fomin, and *Carpinus betulus* var. *typic* Medo, all based upon leaf morphology in the Hyrcanian forest.

The primary objective of our study was to evaluate leaf macro- and micro-morphological variability of *C. betulus* along elevational gradients and to determine leaf variability in the species.

Material and methods

Study site

The current research was conducted in the Hyrcanian forest, northern Iran (Fig. 1). The forest is a wet region; mean annual precipitation varies from 2000 mm in western sites to 600 mm in eastern ones. Mean annual temperature varies from 15°C in western sites to 17.5°C in eastern ones. More than 90% of the region is covered by forest brown soils. Generally, rainfall in the mountain forests is positively associated with, and temperature negatively associated with, increasing elevation (Sagheb Talebi et al. 2003).
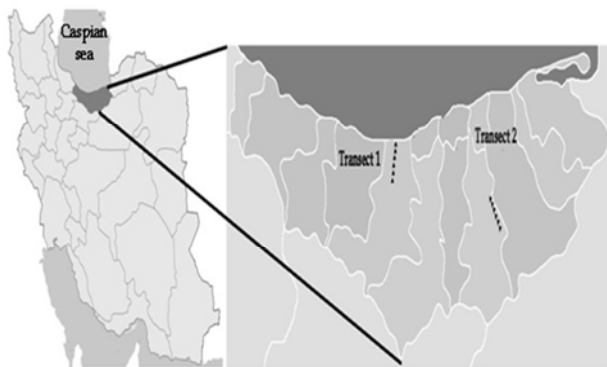
Fig 1. Location of the Hyrcanian forest on a map of Iran (left) and location of the elevational gradient in the Hyrcanian forest (right).

Sampling

Natural populations of *C. betulus* located in two different locations of the Hyrcanian forest were sampled for this study. In each location, we sampled six populations along an altitudinal gradient ranging from 100 m to 1,150 m. We collected 20 fully expanded leaves from the outer (light exposed) part of the crown of 80 trees, resulting in a total collection of 1,600 leaves. Then the leaves of each tree were mixed and 10 leaves were randomly

sampled for analysis. Only leaves lacking signs of abnormal growth, mechanical damage, or pathogen or insect infestation were used.

Macro morphological traits

Eleven leaf macro-morphological traits were used to evaluate the level of leaf variability among the populations. All of the studied leaf traits and their abbreviations are explained in Fig 2. We also evaluated four leaf-character ratios: leaf length/leaf width (LL/LW), leaf length/petiole length (LL/PL), leaf length/distance from leaf base to the widest point (LL/BW), distance from leaf base to the leaf widest point/petiole length (BW/PL). These ratios, which form independent shape variables, have been used extensively in leaf morphometrics (Dichinson et al. 1987; Zarafshar et al. 2010). Leaf area (A, cm2) was also measured with a planimeter. Leaves were then dried at 65°C and weighted on an electric balance for the calculation of leaf mass per area (LMA, mg cm^{-2}).
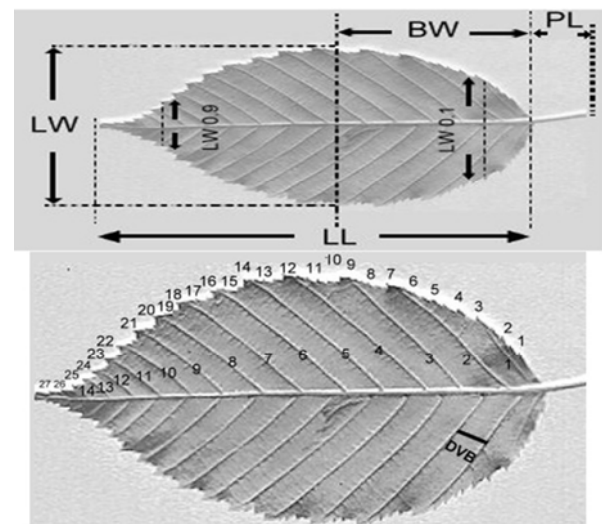
Fig. 2 Presentation of the assessed leaf morphological variables. Lamina length (LL), lamina width measured at the base (LW 0.1) and at the top (LW 0.9), petiole length (PL), distance from leaf base to the leaf maximum width (BW). Number of leaf teeth (T) in bold numbers and number of leaf veins (V) in non bold numbers. DBV is the distance between the first and the second veins.

Micro-morphological traits

In the leaf micro-morphological study, stomatal traits: length (LS), width (WS), area (AS) and density (SD = number/mm^2), were measured. For assessing stomatal traits, five leaves were sampled from each tree. Leaves were boiled for ten minutes, then very thin sub-samples from the bottom surface of the leaves were prepared, with a cutter (Blade). To remove the chlorophyllus effects, the thin samples were soaked in sodium hypochlorite. The epidermal samples were assessed by light microscopy (LM) and photos of stomata were taken with 40X lens. Image Tools software (version 2. 0) was used for stomata morphometrics.

Statistical analysis

After a normality test, the data were log transformed (base of natural logarithms). To generate uncorrelated morphological axis, we performed a Principal Components Analysis (PCA) from the matrix of Loge-transformed tree means. The aim of generating uncorrelated axis was so that any covariance between ecology and morphology along one morphological axis could be interpreted separately from co-variation along another axis (Bruschi 2010). Morphological responses to both regions and elevation were analyzed by performing General Linear Model (GLM), two-way analysis of variance (ANOVA), on the PCA scores with transects by locations as the main effects of the analysis. Then differences among means were determined by Tukey's test. Additionally, a stepwise linear discriminant analysis was employed on all data (using the method of inclusion and removal at each step) to determine the relative importance of characters as discriminators and to assess the degree of separation of different groups by multivariate measurements. A Pearson correlation analysis was used to identify whether the altitudinal gradient of temperature affected PCA scores. All statistical analyses were conducted using the Statistica 6 software package (StatSoft 2001).

Leaf plasticity

Total within-transect plasticity (Pl) was calculated for each parameter using the smallest and greatest mean values Pl = 1 – (x/X), where x is the smallest value and X is the largest value for any given leaf measure (Ashton et al. 1998; Bruschi et al. 2003).

Results

Statistical parameters of leaf traits are presented in Table 1. The mean comparison among the localities clearly shows that trees in the higher elevations have smaller leaf lamina (LL, LW, BW, LW0.1, LW0.9 and A) than those in the lower elevations. In contrast, leaf mass per area (LMA) was high at low altitudes and increased newly at the higher ones. Stomatal dimension was negatively correlated with elevation, while stomatal density was positively correlated with elevation.

Table 1. Mean± standard error of the leaf traits for each locality (bold number belongs to the first transect and non bold belongs to the second transect)

Locality	LL	LW	PL	BW	LW1	LW9	V	DBV	T	LMA
1 (100 m)	8.74 ±0 .37,	4.57± 0.21,	1.25±0.08,	4.23±0.24,	2.385± 0.1,	0.77±0.06,	11.78±0.38,	0.54±0.12,	5.69±0.21,	9.44±6.66,
	10.29±0.76	5.12±0.31	1.48±0.13	4.54±0.42	2.84±.27	1.07±0.65	14.66±0.69	0.56±.52	5.46±0.29	10.94±11.74
2 (350 m)	9.24±0 .35,	4.89± 0.19,	1.62±0.13,	4.51± 0.22,	2.71±0.13,	0.76±0.05,	12.22±0.36,	0.59±0.11,	6.35±0.20,	9.37±1.37,
	11.59±0.76	5.75±0.31	1.34±0.07	5.32±0.42	3.4±.27	0.75±0.06	14.66±0.69	0.58±0.52	6±0.21	11.31±1.88
3 (550 m)	7.72±0 .45,	3.98±0.25,	1.19±0.09,	3.85±0.293,	2.38±0 .17,	0.64±0 .07,	12.2±0.46,	0.86±0.15,	6.04±0.26,	8.24±1.44,
	9.53±0.54	4.65±0.25	1.49±0.97	4.63±0.3	2.54± 0.19	0.78±0.46	13.23±0.49	0.47±0.37	6.7±0.21	9.24±1.26
4 (750 m)	9.31±0 .45,	4.42±0.25,	1.16±0.09,	4.44±0.29,	2.47± 0.17,	0.71±0.07,	12.4±0.46,	0.51±0.15,	5.6±0.26,	7.22±1.29,
	8.87±0.59	4.33±0.24	1.44±0.1	4.15±0.32	2.63±0.21	0.66±0.5	13.4±0.53	0.44±0.04	7.08±0.23	8.49±0.79
5 (950 m)	9.09±0 .42,	4.18±0.23,	1.27±0.09,	4.14±0.27,	2.4±0.16,	0.56± 0.06,	14.0±0.43,	0.46±0.14,	6.0±0.24,	9.06±2.10,
	10.49±0.59	4.61±0.24	1.27±0.61	4.89±0.32	2.39±0.21	0.75±0.5	12.4±.53	0.46±0.4	6.35±0.24	8.99±1.35
6 (1150 m)	8.17±0.45,	3.97±0.25,	1.14±0.09,	3.92±0.29,	2.40±0.17,	0.76±0.07,	12.40±0.46,	0.46±0.15,	5.76±0.26,	11.89±3.79,
	8.25±0.34	3.77±0.34	1.27±0.06	3.67±0.19	2.08±0.12	0.67±0.02	13.41±0.31	0.39±0.2	5.68±0.13	10.58±2.44

Locality	W	A	LLLW	LL/PL	LL/BW	BW/PL	SL	WS	AS	SD
1 (100 m)	256.6±27.4,	27.9±2.2,	1.9±0.05,	7.08±.47,	2.08±.05,	3.41±0.21,	26.18±1.7,	22.63±.91,	472.95±44.41,	239.73±69.21,
	357.4±41.14	33.12±2.9	2±0.12	6.97±.75	2.26±.11	3.08±0.37	26.13±1.1	21.31±.31	244.43±68.85	384.34±42.1
2 (350 m)	232.4±25.9,	24.7±2.1,	1.9±0.05,	7.2±0.4,	2.03±.05,	3.57±0.20,	25.18±1.74,	21.5±1.08,	395.81±44.41,	331.17±69.21,
	313.4±41.14	34.21±2.93	2.05±0.8	7.25±0.75	2.18±.11	3.31±0.37	32.20±1.26	31±1.71	241.36±76.98	395.73±47.15
3 (550 m)	171.5±33.5,	20.8±2.8,	1.9±0.06,	6.6±0.5,	2.05±.07,	3.24±0.26,	26.84±1.74,	20.61±.27,	484.37±44.41,	260.97±69.21,
	211.5±29.09	25.28±2.07	2.05±0.86	6.44±0.53	2.05±.08	3.13±0.26	25.85±1.26	21.06±1.1	228.71±76.98	363.95±47.15
4 (750 m)	235.5±33.5,	31.6±2.8,	2.1±0.67,	8.2±0.5,	2.10±.07,	3.92±0.26,	25.86±1.56,	20.26±.73,	416.75±39.42,	277.19±61.9,
	249±31.8	27.65±2.27	2.05±0.94	7.21±0.58	2.14±.09	3.35±0.28	25.84±1.33	21.55±1.44	210.37±76.98	379.03±47.15
5 (950 m)	222.2±31.0,	24.6±2.5,	2.18±0.06,	7.1±0.5,	2.21±.06,	3.23±0.24,	37.49±1.65,	22±.5,	401.05±39.72,	152.88±61.91,
	290.8±31.8	25.04±2.27	2.27±0.94	7.68±0.58	2.15±.09	3.57±0.28	25.19±1.26	21.9±.20	246.56±68.85	396.75±42.18
6 (1150 m)	263.43±33.56,	21.6±2.8,	2.06±0.06,	7.1±0.5,	2.11±.07,	3.40±0.26,	25.86±1.56,	18.62±.72,	446.01±39.7,	284.38±61.91,
	184.28±18.39	19.14±1.32	2.19±0.05	6.53±0.33	2.28±.05	2.9±0.16	23.87±0.82	22.04±.96	192.91±37.34	429.23±22.8

Note: LL(Lamina length), LW(leaf width), PL(petiole length), BW(distance from leaf base to the widest point), LW.1(lamina width measured at the base), LW.9(lamina width measured at the top), V(number of leaf veins), DBV(the distance between the first and the second veins), T(Number of leaf teeth), LMA(leaf mass per area), W(Leaf weight), A(Leaf area), SL(stomatal length), WS(stomata width), AS(stomatal area), SD(stomata density).

We extracted six principal components explaining about 84.5% of the total variability (Table 2). PC I explained only one fourth of total variance (25.5%), followed by the next five PC's (16.7%, 12.4%, 10.7%, 9.6% and 9.5%, respectively). Most leaf-size parameters, such as lamina length (LL), lamina width (LW), petiole length (PL) and distance from lamina base to the

lamina maximum width (BW) were most strongly correlated with PC I, as expected. Some leaf shape parameters like LL/PL and BW/PL were correlated with PC II. In turn, leaf area (A) and leaf mass per area (LMA) were highly correlated with PC III. Only distance between the first and the second veins (DVB) was significantly correlated with PC IV. The LL/BW ratio was negatively correlated with PC V whereas the only variable positively correlated with PC VI was the LL/LW ratio. Two-way ANOVA on PCI (correlated with leaf size) and on PC III (correlated with leaf weight and area) scores revealed that differences existed between transect and location ($p < 0.05$) (Table 3). Transect and location did not influence leaf-shape; PC2 (correlated with LL/PL and BW/PL) did not differ among transects and locations (Table 4). Post hoc contrasts of differences in PC scores did not show an easily interpretable pattern (Table 5).

Table 2. Factor loadings carried out on macro-morphological data

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
LL	0.932					
LW	0.828					
PL	0.724					
BW	0.896					
DBV				0.763		
LMA			0.934			
A			0.852			
LL/LW						0.873
LL/PL		0.963				
LL/BW					-0.945	
BW/PL		0.917				
Eigenvalue	3.818	2.521	1.836	1.601	1.437	1.427
Explained variance	0.255	0.167	0.124	0.107	0.096	0.095

Note: LL(Lamina length), LW(leaf width), PL(petiole length), BW(distance from leaf base to the widest point), DBV(the distance between the first and the second veins), LMA(leaf mass per area), A(Leaf area).

Table 3. Two-way analysis of variance of leaf stomata data

		SS	df	MS	F	P
Factor1	transect	1.62288	1	1.622882	2.045032	0.158465
	location	2.75743	5	0.551486	0.694941	0.629515
	transect*location	7.85073	5	1.570146	1.978578	0.096595
	Error	42.85293	54	0.793573		

Table 4. Two-way Analysis of Variance carried out on each factor extracted

		SS	df	MS	F	P
Factor1	Transect	6.36023	1	6.360233	9.800678	**
	Location	14.66930	5	2.933860	4.520876	**
	transect*location	19.34682	5	3.869365	5.962423	***
	Error	44.77813	69	0.648958		
Factor2	Transect	4.24166	1	4.241656	4.446139	ns
	Location	2.98919	5	0.597838	0.626659	ns
	transect*location	5.71742	5	1.143483	1.198609	ns
	Error	65.82660	69	0.954009		
Factor 3	Transect	8.13366	1	8.133660	10.74659	**
	Location	10.49579	5	2.099158	2.77351	*
	transect*location	7.34739	5	1.469478	1.94155	ns
	Error	52.22330	69	0.756859		

Table 5. Tukey's Post- hoc summary for comparison of transect and locations in relation to leaf macro and micromorphological traits

transect	location	Macro morphological			Micro morphological
		factor 1	factor2	factor 3	factor 1
1	1	AC	A	AB	AB
1	2	AC	A	A	A
1	3	AB	A	AB	AB
1	4	AC	A	B	AB
1	5	AB	A	BC	AB
1	6	ABC	A	AB	AB
2	1	AB	A	AC	AB
2	2	B	A	A	AB
2	3	AB	A	A	AB
2	4	ABC	A	A	AB
2	5	AB	A	A	AB
2	6	C	A	A	B

When we analyzed micro-morphological data, only one principal component was extracted and this component accounted for more than 63% of variations (Table 6). This component was negatively correlated with stomatal area, large length stomata and stomata index and was positively correlated with stomatal density. Results of ANOVA did not reveal significant differences between transect and localities ($p > 0.05$). Pearson correlations performed on all PC scores showed that only PC I, concerning micro-morphology, was significantly related to the temperature gradient within each transect ($R = -0.43$, $p < 0.05$). Thus, it suggests a significant pattern by which locations from the lower altitudes (higher average temperatures) have bigger stomata and lower stomatal density.

Table 6. Factor loadings carried out on micro-morphological data

| Variable	factor 1
AS	-0.922
SD	0.857
stomata index	-0.952
Eigen value	2.536
Explained variance	0.634

Note: AS(stomatal area), SD(stomata density).

Table 7 shows the most significant characters in the first three canonical discriminant functions, which accounted for 100% of the variations. The first function accounted for 77% of the total variations and separated locations 5 and 6 from the others (Fig. 3). The standardized coefficients of DFA 1 (Discriminant Function Analysis) were highest for vein numbers and petiole length. Function 2 represented another 22% of the total variance and tends to separate the locations within each altitudinal transect. This function was related to tooth number. Function 3 accounted for only 1% of the total variations and was a petiole-length-susceptibility function. Overall misclassification was relatively high (17%) with values of 50%, 100%, 100%, 50%, 100%, and 100% for locations 1, 2, 3, 4, 5 and 6, respectively.

**Table 7. Standardized coefficient from discriminant analysis**

	DFA 1	DFA 2	DFA 3
V	1.998	0.306	-0.173
PL	-1.917	0.354	-0.686
T	0.158	-0.989	-0.338
Eigenvalue	6.134	1.602	0.187
Explained variance	0.770	0.220	0.010

Note: V (number of leaf veins), PL (petiole length), T (Number of leaf teeth).
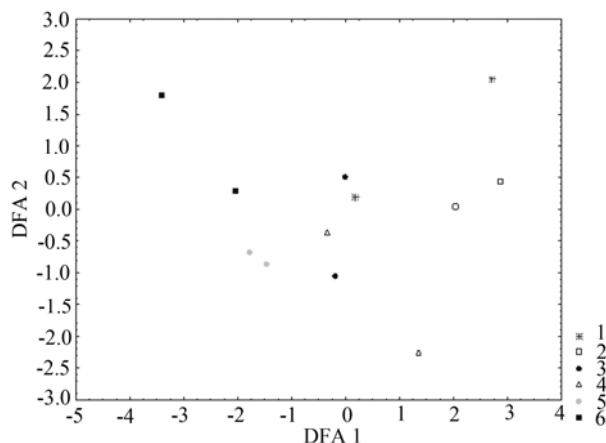
Fig. 3 Scatterplot of the discriminant canonical analysis. Discriminant scores for the first two functions of the means of considered traits for the different localities (1-6) along the two transects are shown on the axis.

Values of leaf plasticity were relatively low for all of the macro-morphological traits (total mean value: 0.51 ± 0.12). Lowest and highest plasticity mean values were recorded for number of veins (V) (Pl = 0.34) and leaf area (A) (Pl = 0.69) respectively. Stomatal traits were not very plastic (total mean value: 0.25 ± 0.09), with the highest value recorded for stomata density (about 0.39). It seems that the two transects showed the same plasticity patterns (Fig. 4).
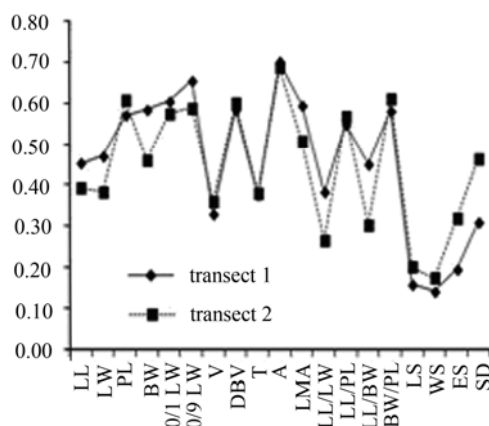
Fig. 4 Plot of plasticity (*Pl*) of macro- and micro-morphological traits for the two analyzed transects. The abbreviations along X-axis refer to Table 1

Discussion

Leaf morphological traits of most woody plants are affected by different abiotic factors along environmental gradients, in particular, several studies documented strong phenotypic variations as adaptive response to altitude (Schoettle and Rochelle 2000; Hovenden and Vander Schoor 2003; Premoli et al. 2007; Yousefzadeh et al. 2010; Bresson et al. 2011). Morphological and physiological adjustments allow individuals to cope with environmental factors associated with elevational gradient: these include dwarfness, compact habit and small, densely pubescent leaves. Our study showed that leaf size in *C. betulus* decreased significantly (Table 1 and Table 2) with increasing altitude in both elevational transects, although this trend was more clear and linear in the second one (Table 1). This result is consistent with the findings of previous studies (Korner et al. 1986; Zhang and Marshall 1995). Yousefzadeh et al. (2010) observed a significant effect of altitude on width of lamina, base angle, number of paired leaf veins in *Parrotia persica* sampled in the Hyrcanian forest. In the same area, Akbarian et al (2011) reported significant and negative correlation values of leaf blade, petiole length and stomatal length with increasing elevation on Alnus subcordata. These morphological variations among leaves appear to be a consequence of adaptive strategies to abiotic factors along elevational gradients, such as soil moisture (Beerling et al. 1996), air temperature (Panek and Waring 1995) and atmospheric CO_2 concentrations (Ehleringer and Cerling 1995; Marshall and Monserud 1996). We also found that LMA was higher at lower and higher altitudes but decreased at mid altitudes. The higher LMA probably results from higher temperatures and summer drought at lower altitudes (Sletvold and Ågren 2011) and cold temperature at higher altitudes (Kao and Chang 2001; Bresson et al. 2011). In a large scale study of the Catalan Q. ilex forests (Ogaya and Peñuelas 2007), higher LMA was observed in drier environments, but also in more sunny areas and with lower air temperatures. The effect of air temperature was even greater than the effect of water availability, especially in the case of cold winter temperature that was considered as the most important factor affecting LMA values in this species. On the other hand, it has been pointed out that leaves with high LMA have a structure that reduces photosynthetic rates but also water losses by transpiration (Hultine and Marshall 2000).

Our results showed an increase in stomatal density with increasing altitude although this increase was not linear along the altitudinal gradients (Table 1). Previous studies relating stomatal density to altitude obtained mixed results. Some found that stomatal density increases linearly with altitude (Körner and Cochrane 1985), that it increases and then decreases again (Körner et al. 1989), decreases with altitude (Schoettle and Rochelle 2000) or remains unchanged (Woodward 1986; Holland and Richardson 2009). An increase in SD might be associated to an improved efficiency in carbon dioxide uptake (McElwain 2004), whereas a decrease in SD might reflect a conserving water strategy (Schoettle and Rochelle 2000). Li et al. (2006)

showed that stomatal area, stomatal length and index of Q. aquifolioides decreased with increasing altitude above 2800 m although leaf morphological and physiological responses to altitudinal gradients were non-linear with increasing altitude. Of the ecological variables they investigated, increasing drought from lower to higher altitudes was considered the main stress force. The authors concluded that colder soils could reduce water uptake of root system and induce water stress. Significant Spearman rank correlation was observed between temperature and PC performed for stomatal traits, suggesting that micro-morphological analysis could be a useful tool to analyze phenotypic adaptation in this species. Moreover, this result could be interesting from the perspective of paleontological reconstruction of altitude (Kouwenberg et al. 2007). In general, different environments that represent different microclimates influence the variation of leaf quantitative characters (Valladares et al. 2002). For example, Bruschi et al. (2003) and Bruschi (2010) reported lower values of leaf plasticity for xeric populations of *Quercus petrea*. Although central and eastern parts of the Hyrcanian forest do not experience the same climate, we found similar patterns of plasticity for each transect. Number of veins, number of teeth and stomatal dimensions showed the least plasticity, while leaf area and stomatal density showed the highest plasticity. Some studies showed that vein density were less sensitive to changes in environmental conditions, suggesting that leaf vein traits are conservative (Sack et al. 2008; Zhu et al. 2011). Vein traits are considered to reflect the gas and water change characteristics between the leaves and the atmosphere, which are strongly affected by climate (Zhu et al. 2011). It seems, however, that each species showed a special pattern in leaf plasticity. Zarafshar et al. (2010), for example, reported the highest plasticity for leaf petiole length of Castanea sativa and Akbarian et al (2011) reported the highest plasticity for leaf-apex length of *Alnus subcordata*. On the other hand, leaf traits that show the greatest plastic response are most important for leaf functioning in different environments (Markesteijn et al. 2007). If this is the case, leaf area, leaf mass per area and stomatal density could influence altitudinal acclimation of *Carpinus betulus* to climate change (Aitkin et al. 2008). To understand adaptation of this species to altitude, more large-scale investigations are needed, also taking ecophysiological traits into account. Moreover, common garden experiments will be required to understand the environmental and genetic components of variation (Bresson et al. 2011).

References

Aitkin SN, Yeaman S, Holliday JA, Wang TL, Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications*, **1**: 95–111.

Akbarian MR, Tabari M, Akbarinia M, Zarafshar M, Meave JA, Yousefzadeh H, Satarian A. 2011. Effected of elevation gradient on leaf and stomata morphology of Caucasian alder (*Alnus subcordata*) in Hyrcanian forests (Iran). *Journal of Folia Oecologica*, **38**: 1–7.

Ashton PMS, Olander LP, Berlyn GP, Thadani R, Cameron IR. 1998. Changes in leaf structure in relation to crown position and tree size of *Betula papyrifera* within fire origin stands of interior cedar-hemlock. *Canadian Journal of Botany*, **76**: 1180–1187.

Austrheim G. 2002. Plant diversity patterns in semi-natural grasslands along an elevational gradient in southern Norway. *Plant Ecology*, **161**: 193–205.

Beerling DJ, Heath J, Woodward FI, Mansfield TA. 1996. Interactions in trees: observations and mechanisms. *New Phytologist*, **134**: 235–242.

Bresson, CC, Vitasse Y, Kremer A, Delzon S. 2011. To what extent is altitudinal variation of functional traits driven by genetic adaptation in European oak and beech. *Tree Physiology*, **31**: 1164–1174.

Bruschi P. 2003. Within- and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations. *Trees*, **17**: 164–172.

Bruschi P. 2010. Geographical variation in morphology of *Quercus petraea* (Matt.) Liebl. as related to drought stress. *Plant Biosystems*, **2**: 298–307.

Dichinson TA. 1987. Another approach to leaf shape comparisons. *Taxon*, **36**: 1–20.

Ehleringer JR, Cerling TE. 1995. Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 levels in plants. *Tree Physiology*, **15**: 105–111.

Geeske J. 1994. Leaf morphology along environmental gradients in Hawaiian Metrosideros polymorpha. *Biotropica*, **26**: 17–22.

Holland N, Richardson AD. 2009. Stomatal length correlates with elevation of growth in four temperate species. *Journal of Sustainable Forestry*, **28**: 63–73.

Hovenden MJ, Vander Schoor JK. 2003. Nature versus nurture in the leaf morphology of Southern Beech, Nothofagus cunninghamii (Nothofagaceae). *New Phytologis*, **131**: 585–594.

Hultine KR, Marshall JD. 2000. Altitude trends in conifer leaf morphology and stable carbon isotope composition. *Oecologia*, **123**: 32–40.

Kao W, Chang K. 2001. Altitudinal trends in photosynthetic rate and leaf characteristics of *Miscanthus* populations from central Taiwan. *Australian Journal of Botany*, **49**: 509–514.

Körner C. 1999. Alpine plant life: functional plant ecology of high mountain ecosystems. Springer. Berlin Heidelberg, New York. p. 344.

Körner C. 2007. The use of altitude in ecological research. *Trends in Ecology & Evolution*, **22**: 567–574.

Körner C, Cochrane PM. 1986. Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevational gradient. *Oecologia,* **66**: 443–455.

Körner C, Bannister P, Mark AF. 1986. Altitudinal variation in stomatal conductance, nitrogen content and leaf anatomy in different plant life forms in New Zealand. *Oecologia*, **69**: 577–588.

Körner C, Neumayer M, Menendez-Riedl S, Smeets-Scheel A. 1989. Functional morphology of mountain plants. Flora, **182**: 353–383.

Kouwenberg LLR, Kürschner WM, McElwain JC. 2007. Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry. *Mineralogy and Geochemistry*, **66**: 215–241.

Li S, Assmann SM, Albert R. 2006. Predicting essential components of signal transduction networks a dynamic model of guard cell abscisic acid signaling. *PLOS Biology*, 4:e312. Doi: 10.1371/journal. pbio.0040312.

Lomolino MV. 2001. Elevation gradients of species diversity: historical and

prospective views. – Glob. *Ecology and Biogeography*, **10**: 3−13.

Markesteijn L, Poorter L, Bongers F. 2007. Light-dependent leaf trait variation in 43 tropical dry tree species. *American Journal of Botany*, **94**: 515−525.

Marshall JD, Monserud RA. 1996. Homeostatic gas-exchange parameters inferred from 13C/12C in tree rings of conifers. *Oecologia*, **105**: 13–21.

McElwain JC. 2004. Climate-independent paleoaltimetry using stomatal density in fossil leaves as proxy for CO_2 partial pressure. *Geology*, **32**: 1017–1020.

Ogaya R, Peñuelas J. 2007. "Leaf mass per area ratio in *Quercus ilex* leaves under a wide range of climatic conditions. The importance of low temperatures". *J Acta Oecologica,* **31**: 168−173.

Panek JA, Waring RH. 1995. Carbon isotope variation in Douglas-fir foliage: improving the δ13C–climate relationship. *Tree Physiology*, **15**: 657.

Premoli AC, Rafafele E, Mathiasen P. 2007. Morphological and phenological differences in *Nothofagus pumilio* from contrasting elevations: Evidence from a common garden. *Austral Ecology*, **32**: 515−523.

Roderick ML, Berry SL, Noble IR. 2000. A framework for understanding the relationship between environment and vegetation based on the surface area to volume ratio of leaves. *Functional Ecology.* **14**: 423−437.

Sabety H. 2001. *Forestry, tree and shrubs of iran.* Yazad university. p. 791.

Sack L, Crowan PD, Holbrook NM. 2003. The major veins of mesomorphic leaves revisited: tests for conductive overload in *Acer saccharum* (Aceraceae) and *Quercus rubra* (Fagaceae). *American Journal of Botany*, **90**: 32−39.

Sagheb-Talebi KH, Schuetz JPH, Aas G. 2003. Influence of some site conditions on qualitative characteristics of beech saplings. *Iranian Journal of Natural Resources*, **55**: 505−520.

Schoettle AW, Rochelle SG. 2000. Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevations. *American Journal of Botany*, **87**: 1797−1806.

Sletvold N, Agren J. 2012. Variation in tolerance to drought among Scandinavian populations of *Arabidopsis lyrata*. *evolutionary ecology*, **26**: 559−577.

Valladares F, Balaguer I, Martiunez-Ferri E, Perez-Corona E, Manrique E. 2002. Plasticity, instability and canalization: Is the phenotypic variation in seedlings of sclerophyll oaks consistents with the environmental unprudectability. *New Phytologist*, **156**: 457−467.

Wang GH, Zhou GS, Yang LM, Li ZQ. 2003. Distribution, species diversity and life-form spectra of plant communities along an altitudinal gradient in the northern slopes of Qilianshan Mountains, Gansu China. *Plant Ecology.* **165**: 169−181.

Woodward FI. 1986. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia*, **70***:* 580–586.

Yousefzadeh H, Tabari M, Akbarinia M, Akbarian MR, Bussotti F. 2010. "Morphological plasticity of *Parrotia persica* leaves in eastern Hyrcanian forests (Iran) is related to altitude. *Nordic Journal of Botany*, **28**: 344−349.

Zarafshar M, Akbarinia M, Bruschi P, Hosseiny SM, Yousefzadeh H, Taieby M, Sattarian A. 2010. Phenotypic variation in chestnut (*Castanea sativa* Mill.) natural populations in Hyrcanian forest (north of Iran), revealed by leaf morphometrics. *Folia Oecologica*, **37**: 113−121.

Zhang JW, Marshall JD. 1995. Variation in carbon isotope discrimination and photosynthetic gas exchange among populations of *Pseudotsuga menziesii* and *Pinus ponderosa* in different environments. *Functional Ecology*, **9**: 402−412.

Zhu YH, Kang HZ, Xie Q, Wang Z, Yin S, Liu CJ. 2011. Pattern of leaf vein density and climate relationship of *Quercus variabilis* populations remains unchanged with environmental changes. *Trees*, **26**(2): 597−607.